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1. REPORT DATE (DD-MM-YYYY) 28-02-2003		2. REPORT TYPE Final Technical Report		3. DATES COVERED (From - To) 01-01-97 - 31-12-02	
4. TITLE AND SUBTITLE Receptors, Afferent Signaling and Central Integration in Compensated and Uncompensated Hemorrhagic Shock				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER N00014-97-1-0145	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Johnson, Alan Kim				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Iowa Iowa City, IA 52242				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) University of Iowa Iowa City, IA 52242				10. SPONSORING/MONITOR'S ACRONYM(S)	
				11. SPONSORING/MONITORING REPORT NUMBER	
12. DISTRIBUTION/AVAILABILITY STATEMENT Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT An understanding of the central and peripheral mechanisms which regulate sympathetic outflow during acute blood volume loss is critical for developing therapeutic treatments for hemorrhagic shock. Insight into the mechanisms by which melanopeptides, LV-HTS, and serotonergic drugs facilitate sympathetic outflow during hemorrhage may lead to the development of pharmacological interventions for hemorrhagic shock. Recent experiments conducted in our laboratory provide important new information about the saluretic effects and mechanisms of 3 potential therapeutic strategies to prevent or reverse phase II shock. We have 1) discovered that $\gamma_2$ -MSH when administered systemically promotes survival, 2) confirmed that LV-HTS administered systemically improves survival and that this treatment is even more effective when administered directly to the brain cerebrospinal fluid, and 3) treatment with a frequently used serotonergic (5-HT) drug, the 5-HT selective reuptake inhibitor, fluoxetine, enhances baroreceptor reflex gain which may contribute to enhanced survival after severe hypotensive hemorrhage.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  U	18. NUMBER OF PAGES  9	19a. NAME OF RESPONSIBLE PERSON Johnson, Alan Kim
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (Include area code) 319-335-2423

20030305 083

STANDARD FORM 298 (Rev. 8-98)  
Prescribed by ANSI Std. Z39-18

## FINAL TECHNICAL REPORT

GRANT #: N00014-97-1-0145

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INSTITUTION: University of Iowa

GRANT TITLE: Receptors, Afferent Signaling and Central Integration in Compensated and Uncompensated Hemorrhagic Shock

AWARD PERIOD: 1 January 1997 - 31 December 2002

OBJECTIVE: A comprehensive understanding of how the brain controls sympathetic and endocrine outflow during compensated (phase I) vs. early uncompensated (phase II) shock will lead to the development of better therapeutic treatments for severe hypotensive hypovolemia. Our research program has pursued two specific objectives that will increase basic knowledge about the neural control of the circulation in shock. The first was to define the hormonal and afferent nervous system signaling pathways over which systemic treatments with agents such as melano peptides or low volume (LV) hypertonic saline (HTS) act to effect therapeutic resuscitation from hemorrhagic shock. The second was to define the critical central sites and brain neurochemical systems that control the pattern of autonomic and endocrine outflow mediating recovery and resuscitation from hemorrhagic shock.

APPROACH: This research used models of hemorrhage-induced shock in the rat combined with or without volemic resuscitation. The efficacy and mechanisms of melano peptides, LV-HTS, and serotonergic drugs in reversing phase II hemorrhagic shock and promoting survival (i.e., 24 and 72 hr post-hemorrhage) were investigated by administering the putative therapeutic treatments either centrally or systemically.

### ACCOMPLISHMENTS:

A. The Effects of Systemic  $\gamma_2$ -Melanocyte Stimulating Hormone ( $\gamma_2$ -MSH) and LV-HTS and on Regional Hemodynamics after Hypertensive Hemorrhage. Work conducted in our laboratory and elsewhere suggests that  $\gamma_2$ -MSH and LV-HTS, respectively both significantly increase arterial blood pressure to enhance survival following severe hypotensive hemorrhage. We have completed experiments to determine whether these treatments have their effects during hypotensive hemorrhage by activating similar efferent mechanisms. That is, we examined the effects of graded doses of  $\gamma_2$ -MSH and of LV-HTS in rats subjected to uncompensated hemorrhagic shock on hemodynamics in key vascular beds.

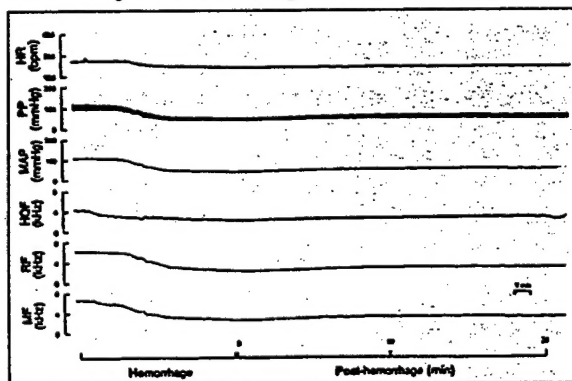


Fig. 1: Typical cardiovascular and hemodynamic changes accompanying blood withdrawal (1-1.5 ml/min) to induce phase II hypovolemic shock.

Male Sprague-Dawley rats (250-350 g) were anesthetized with pentobarbital (50 mg/kg) and implanted with femoral artery and venous catheters and Doppler flow probes on the mesenteric artery, one renal artery and the terminal aorta. Rats were then hemorrhaged (1-1.5 ml/min) via the femoral artery until arterial pressure was <40 mmHg. The mean arterial pressure (MAP) of control animals remained at this low level and there was a sustained bradycardia and marked reductions in blood flow in the hindquarter, renal and mesenteric beds throughout a 20 min post-hemorrhage period (Fig. 1). Animals receiving

bolus intravenous injections of 10, 20 and 40  $\mu$ g/kg of  $\gamma_2$ -MSH showed short-lived but significant increases in MAP (Fig. 2a,b). All doses of  $\gamma_2$ -MSH increased heart

rate (Fig. 2a,b). Increases in mesenteric (MR) and hindquarter (HQR) resistances (or decrease in flow) were seen with the two largest doses and renal resistance (RR) increased with the largest dose of the peptide (Fig. 2c). Interestingly, in contrast to  $\gamma_2$ -MSH, treatment with 0.75, 1.5, and 3.0 mmol/kg of HTS

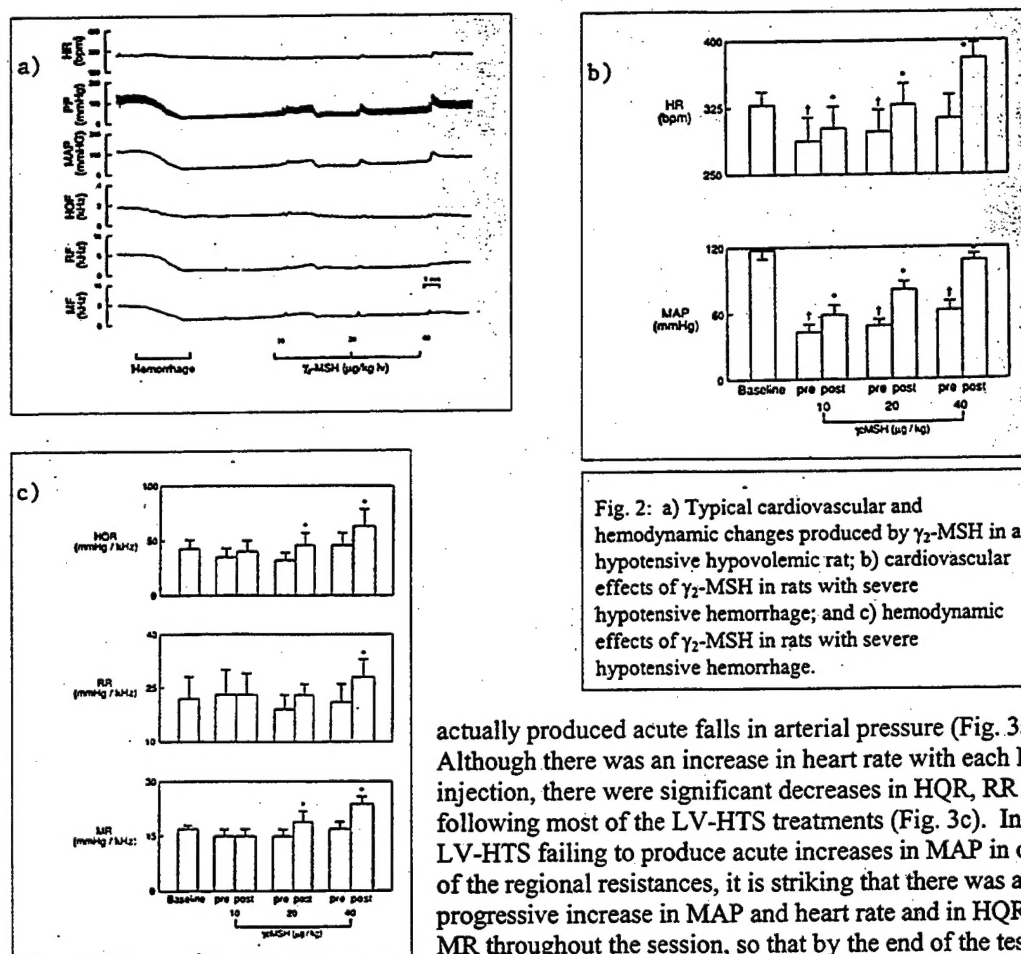


Fig. 2: a) Typical cardiovascular and hemodynamic changes produced by  $\gamma_2$ -MSH in a hypotensive hypovolemic rat; b) cardiovascular effects of  $\gamma_2$ -MSH in rats with severe hypotensive hemorrhage; and c) hemodynamic effects of  $\gamma_2$ -MSH in rats with severe hypotensive hemorrhage.

actually produced acute falls in arterial pressure (Fig. 3a,b.). Although there was an increase in heart rate with each LV-HTS injection, there were significant decreases in HQR, RR and MR following most of the LV-HTS treatments (Fig. 3c). In spite of LV-HTS failing to produce acute increases in MAP in one or more of the regional resistances, it is striking that there was a slow, progressive increase in MAP and heart rate and in HQR, RR and MR throughout the session, so that by the end of the test none of

the parameters were significantly less than baseline values (Fig. 3b,c). In fact, MR was significantly increased at the conclusion of the period.

Taken together, the results of these experiments indicate that hemodynamic changes induced by treatments having putative therapeutic effects during phase II hemorrhagic shock produced markedly different changes in cardiovascular parameters and regional vascular resistances. This suggests that salutary actions of these agents involve different mechanisms. In light of this possibility, it is likely that combined administration of  $\gamma_2$ -MSH and LV-HTS will produce synergistic actions to restore the perfusion of key vascular beds and facilitate recovery from hemorrhagic shock.

In more recent work, we have focused on using lower concentrations of HTS. We have studied the hemodynamic effects of 5% NaCl because of its current FDA approval. In pentobarbital anesthetized rats instrumented with Doppler flow probes on the mesenteric and renal arteries and the terminal aorta, bolus (10% of the shed blood volume) administration of 5% NaCl produces a brief biphasic (minimal pressor) response and then a gradual, progressive rise in resistance in the mesenteric, renal and hindquarter vascular beds and a rise in MAP (Figure 4). The results are similar to those we previously observed and with highly concentrated HTS (~20%).

$\alpha$ -MSH is another melanocortin peptide structurally-related to  $\gamma_2$ -MSH and one which is approved for administration to humans. Male Sprague-Dawley rats (300-325 g) were instrumented with carotid and femoral arterial and femoral venous catheters 48 hrs prior to testing. At the time of testing, the animals were placed in hanging metabolic cages and vascular lines were connected to measure blood pressure, induce hemorrhage and deliver treatments. After adaptation and acquisition of baseline blood pressure

recordings, a 3.6 ml/100 g-body weight (bw) hemorrhage was initiated. Blood withdrawal typically took about 10 min and lowered MAP from approximately 120 to 50 mmHg. Immediately upon conclusion of the hemorrhage, experimental animals received 8.25, 16.5 or 27.5  $\mu$ g of  $\alpha$ -MSH administered intravenously; control animals received isotonic saline in comparable volumes to those administered to the

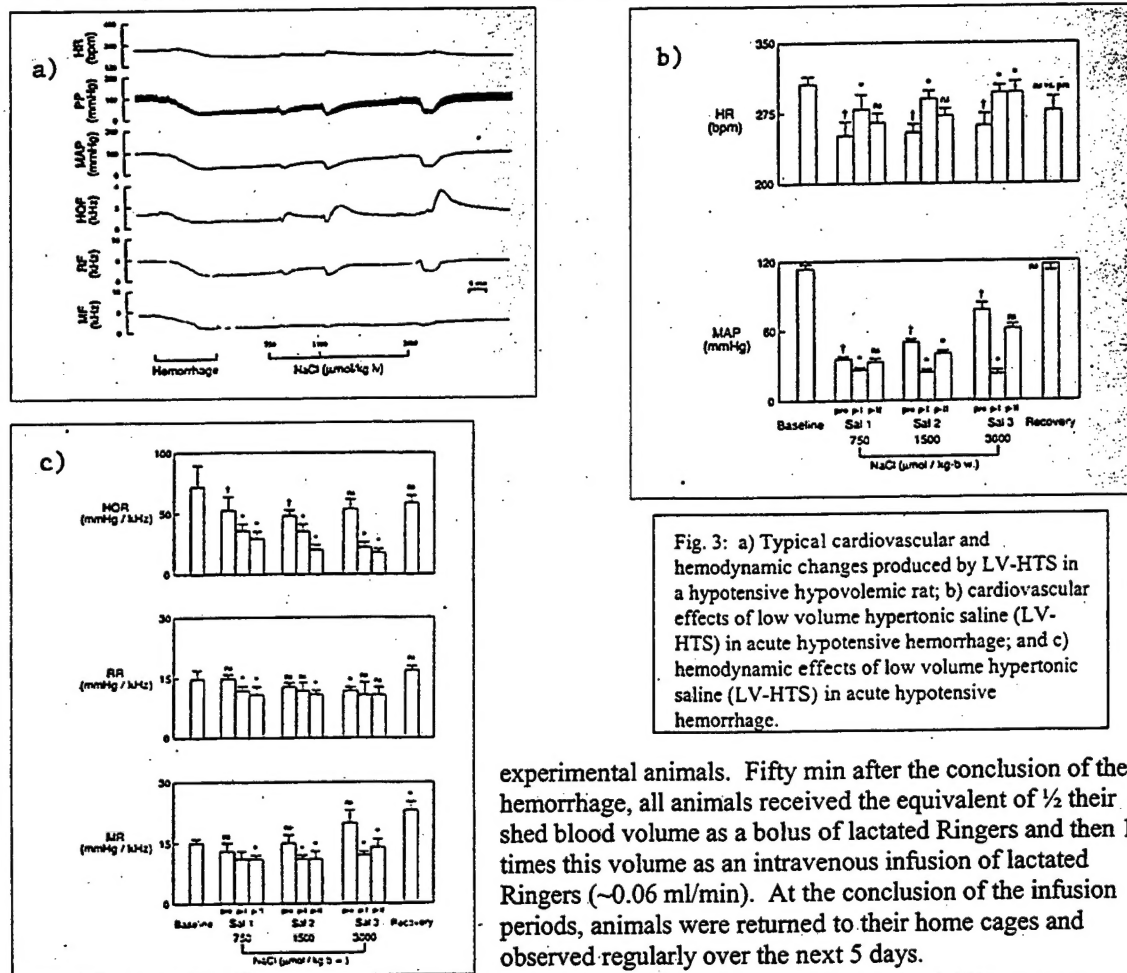


Fig. 3: a) Typical cardiovascular and hemodynamic changes produced by LV-HTS in a hypotensive hypovolemic rat; b) cardiovascular effects of low volume hypertonic saline (LV-HTS) in acute hypotensive hemorrhage; and c) hemodynamic effects of low volume hypertonic saline (LV-HTS) in acute hypotensive hemorrhage.

experimental animals. Fifty min after the conclusion of the hemorrhage, all animals received the equivalent of  $\frac{1}{2}$  their shed blood volume as a bolus of lactated Ringers and then  $1\frac{1}{2}$  times this volume as an intravenous infusion of lactated Ringers ( $\sim 0.06$  ml/min). At the conclusion of the infusion periods, animals were returned to their home cages and observed regularly over the next 5 days.

Summarized in Table 1 are the effects of acute isotonic saline (control) and  $\alpha$ -MSH treatments on survival of hypotensive hemorrhage. Nearly all animals died following acute isotonic saline treatment (controls; 7% survival). In contrast, 8.25 and 16.5  $\mu$ g intravenous treatments of  $\alpha$ -MSH promoted survival in 4 out of 8 (50% survival) and 8 out of 14 (57% survival) animals, respectively. Treatment with 27.5  $\mu$ g of  $\alpha$ -MSH however appears to be on a declining limb of a dose response curve. The results of these experiments suggest that  $\alpha$ -MSH should be explored further for its salutary effects in the treatment of severe hypotensive hemorrhage.

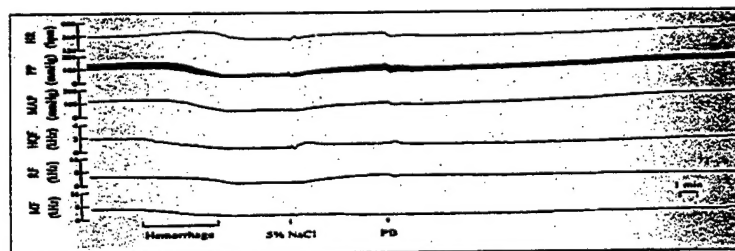


Fig. 4

Table 1: The effects of  $\alpha$ -MSH treatment immediately post-hemorrhage (3.6 ml/100 g-bw) on hemorrhagic shock.

	<u>Controls (Saline)</u>	<u>8.25 <math>\mu</math>g</u>	<u>16.5 <math>\mu</math>g</u>	<u>27.5 <math>\mu</math>g</u>
Died prior to reperfusion	13	4	6	4
Survived 5 days post-resuscitation	<u>1</u>	<u>4</u>	<u>8</u>	<u>0</u>
TOTAL	14	8	14	4

We found that animals subjected to our severe hemorrhage shock model (3.6 ml/100 g-bw blood withdrawal over 10 min in 300-325 g-bw male rats) that were treated with 10  $\mu$ l of 0.33 M NaCl into the lateral cerebral ventricle followed by a 3  $\mu$ l/min for approximately 10-15 min of 0.33 M NaCl produced a profound increase in arterial pressure (i.e., an increase of 65-76 mmHg). The increase in pressure was markedly greater than the typical transient increase of 20-30 mmHg seen in control rats. Based on these observations, we decided to attempt to optimize the central infusion conditions. We tested a total of 24 hemorrhaged animals with lateral ventricle administration of isotonic saline (control) vs. 0.33 M NaCl at rates of 1 to 3  $\mu$ l/min following a 10  $\mu$ l lateral ventricle bolus for time periods of 15 to 50 min. Survival rates for experimental animals treated with lateral ventricle-infused 0.33 M NaCl for these longer periods were increased. However, we noted that many of the 0.33 M NaCl lateral ventricle infused animals show signs of seizures. Consequently, we conducted a subsequent study in which the concentration of NaCl in the experimental group was reduced to 0.27 M NaCl. Specifically, control (n=9) and experimental (n=10) rats received 3.6 ml/100 g-bw hemorrhages over approximately 10 min. At the conclusion of the hemorrhage, animals received a 10  $\mu$ l bolus followed by a 50 min lateral ventricle infusion (3  $\mu$ l/min) of either 0.15 M (control) or 0.27 M NaCl. Nine out of 10 experimental animals survived 1 hr post hemorrhage vs. only 3 out of 9 of the control animals ( $\chi^2 = 13.07$ ;  $P \leq 0.001$ ). At approximately 60 min after hemorrhage, half of the shed blood volume was administered as Ringer's lactate. All rats survived 1 hr after the reinfusion and 8 out of 9 experimental and 3 out of 3 controls survived 2 hr after reinfusion. These results suggest that activation of the sympathetic nervous system by a remarkably small amount of HTS administered directly to the brain reverses the acute effects of severe hypotensive hemorrhage to promote survival.

In order to determine if lateral ventricular infused HTS is more effective than the same concentration and volume of HTS administered systemically in facilitating survival from severe hypotensive hemorrhage, a second experiment was conducted. Animals were prepared with catheters and hemorrhaged 2 days later as previously described. After removal of 3.6 ml/100 g-bw, a 10  $\mu$ l bolus of 0.27 M NaCl was administered over 1 min and then at a rate of 3  $\mu$ l/min for 50 min. At the conclusion of the 50 min infusion  $\frac{1}{2}$  the volume of the shed blood was administered as Ringers lactate. Control animals received the same procedure except they received isotonic saline. Five of 15 rats receiving intravenous HTS survived whereas all of the 12 animals receiving isotonic saline died within 1 hr after the start of hemorrhage. The survival rate for the intracerebroventricular infusions of HTS was 90% whereas the survival rate for intravenous infusions of HTS was only 33%. The results support the interpretation that LV-HTS therapy for hemorrhagic shock probably exerts its salutary effects through a CNS action to enhance the pattern of systemic hemodynamics.

**B. Serotonergic Mechanisms and Protection Against Hypovolemic Hemorrhage.** In previous studies (Scrogin, Veelken and Johnson, 1998)<sup>1</sup>, we found that central administration of methysergide, a serotonergic drug, that acts primarily as a 5-HT<sub>1/2</sub> receptor antagonist but which also has some 5-HT<sub>1A</sub> receptor agonist activity, significantly delays the withdrawal of renal sympathetic tone and bradycardia during non-compensated (phase II) hemorrhagic shock. We have completed work showing that intracerebroventricular administration of the highly selective 5-HT<sub>1A</sub> receptor agonist, ( $\pm$ )-8-hydroxy-dipropylaminotetralin (8-OH-DPAT), also produces the same delay in onset of bradycardia and withdrawal of renal sympathetic nerve activity during hemorrhage. This finding suggests two interpretations: first,

<sup>1</sup> Scrogin, K. E., Veelken, R., & Johnson, A. K.: Central methysergide prevents renal sympathoinhibition and bradycardia during hypotensive hemorrhage. *Am. J. Physiol.*, 1998, 274, H43-H51.



that these drugs are blocking 5-HT activity possibly through methysergide acting on post-synaptic receptors (perhaps in the raphe nuclei) and through 8-OH-DPAT's classic action on somatic/dendritic autoreceptors (i.e., in the hindbrain); or alternatively, that methysergide is exerting a 5-HT inhibitory action by acting primarily as an agonist on 5-HT<sub>1A</sub> autoreceptors. In either case, it appears that acute pharmacological manipulations that reduce activity in central 5-HT pathways block the autonomic changes accompanying phase II of hemorrhagic shock.

These observations made with the 5-HT<sub>1A</sub> receptor agonist prompted us to ask what types of systemically administered, clinically employed 5-HT-related drugs are likely to enhance cardiovascular function? We decided to test the effects of the widely used anti-depressant serotonin selective reuptake inhibitor, fluoxetine (Prozac®), to see if we could enhance the sympathetic baroreceptor reflex. We examined the effects of 4 days of fluoxetine administration (10 mg/kg, daily, i.p.) on baroreceptor reflex control of lumbar sympathetic nerve activity (LSNA). As compared to control (vehicle; 0.9% saline) administration, fluoxetine did not significantly change resting MAP or heart rate. However, it did significantly enhance the maximum baroreflex elicited increases in LSNA (VEH:  $352 \pm 26$  vs. fluoxetine  $454 \pm 41$ ; % baseline LSNA;  $P < 0.05$ ) and increased the maximum reflex gain (vehicle:  $-7.5 \pm 1.0$  vs. fluoxetine  $-12.8 \pm 2.3$ ; % LSNA/mmHg;  $P < 0.05$ ).

We also tested 4 day fluoxetine (10 mg/kg/day) treatment under a condition known to blunt cardiovascular baroreceptor reflexes, cardiovascular deconditioning. We used a hindlimb suspension paradigm which models the effects of cardiovascular deconditioning induced by bed rest or by exposure to microgravity. Fluoxetine treatment also enhanced LSNA baroreceptor reflexes in rats that had undergone 14 days of hindlimb unloading before testing.

	Hindlimb Unweighted	
	Control	Fluoxetine Treated
Maximum Reflex Gain % LSNA	$252 \pm 5.2$	$331 \pm 6$

**CONCLUSIONS:** Recent experiments conducted in our laboratory provide important new information about the saluretic effects and mechanisms of 3 potential therapeutic strategies to prevent or reverse phase II shock. We have 1) discovered that  $\gamma_2$ -MSH when administered systemically promotes survival, 2) confirmed that LV-HTS administered systemically improves survival and that this treatment is even more effective when administered directly to the brain cerebrospinal fluid, and 3) treatment with a frequently used serotonergic (5-HT) drug, the 5-HT selective reuptake inhibitor, fluoxetine, enhances baroreceptor reflex gain which may contribute to enhanced survival after severe hypotensive hemorrhage.

**SIGNIFICANCE:** An understanding of the central and peripheral mechanisms which regulate sympathetic outflow during acute blood volume loss is critical for developing therapeutic treatments for hemorrhagic shock. Insight into the mechanisms by which melanopeptides, LV-HTS, and serotonergic drugs facilitate sympathetic outflow during hemorrhage may lead to the development of pharmacological interventions for hemorrhagic shock.

**PATENT INFORMATION:** None

**AWARD INFORMATION:** Appointed as F. Wendell Miller Distinguished Professor

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#### BOOK CHAPTERS, SUBMISSIONS, AND ABSTRACTS

1. Vasquez, E. C., & Johnson, A. K.: Green fluorescent protein as a reporter of adenovirus-mediated gene transfer and expression in the hypothalamic-neurohypophyseal system. In: B.W. Hicks (ed.), Methods in Molecular Biology, Vol. 183: Green Fluorescent Protein: Applications and Protocols, Totowa: Humana Press, 2002, 321-329.
2. Moffitt, J. A., & Johnson, A. K.: Short-term fluoxetine treatment enhances baroreflex control of sympathetic nervous system activity. (submitted).
3. Grippo, A. J., Santos, C. M., Johnson, R. F., Beltz, T. G., Martins, J. B., Felder, R. B., & Johnson, A. K.: Increased susceptibility to ventricular arrhythmias in an experimental rodent model of depression. (submitted).
4. Whyte, D. G., Thunhorst, R. L., & Johnson, A. K.: Reduced thirst in old, thermally dehydrated rats. (submitted).